Two new species of Fusarium: Fusarium brevicatenulatum from the noxious weed Striga asiatica in Madagascar and Fusarium pseudoanthophilum from Zea mays in Zimbabwe

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Abstract: Two new species are described and illustrated: Fusarium brevicatenulatum isolated from the noxious witchweed (Striga asiatica) in Madagascar, and F. pseudoanthophilum isolated from Zea mays in Zimbabwe. F. brevicatenulatum is characterized by long-oval to obovoid, mostly 0-septate conidia adhering usually in false heads on mostly monophialidic conidiophores in the aerial mycelium, the formation of very short false chains of conidia under continuous black light, the rare production of 3-septate sporodochial conidia, and the absence of chlamydospores; F. pseudoanthophilum by the production of mostly 0-septate, obovoid to clavate and some pyriform conidia that adhere in false heads and sometimes in very short chains on conidiophores of the aerial mycelium that are often branched and polyphialidic, by 3-5-septate sporodochial conidia, and by chains of chlamydospores.

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INTRODUCTION

Parasitic weeds of the genus Striga (Scrophulariaceae) are an increasing problem in crop production in the savannah regions of Africa. S. hermonthica (Del.) Benth. and S. asiatica (L.) Kuntze are parasitising cereals such as maize (Zea mays L.), sorghum (Sorghum bicolor (L.) Moench) and millet (Pennisetum americanum L.), while S. gesnerioides (Willd.) Vatke primarily attacks cowpea (Vigna unguiculata (L.) Walp.) (Sauerborn, 1991). Striga asiatica was probably introduced from the African continent to Madagascar several years ago where it has been spreading in the Middle West of this country. Striga asiatica currently infests maize and upland rice (Oryza sativa L.) and is considered by farmers as the most important constraint to cereal production (Geiger et al., 1996). Because of the complexity of the host-parasite interaction, benefits of most of the control strategies such as crop rotation, hand-pulling or the use of herbicides become known only after a period of several years. An integrated approach is needed which might include natural antagonists as biocontrol agents. Fusarium nygamai Burgess & Trimboli, collected in Sudan in 1989, and F. oxysporum Schlecht., collected in Ghana from diseased S. hermonthica plants, were found to reduce S. hermonthica incidence by more than 90% in in vitro experiments (Abbasher and Sauerborn, 1992; Abbasher et al., 1995; Kroschel et al., 1996). In Spring 1994, an additional survey was conducted in the Middle West of Madagascar in the central highlands at an elevation between 700 and 1000 m above sea level to identify native fungi associated with S. asiatica. Fusaria were found among the isolates and these were sent to the Federal Biological Research Centre (BBA) in Berlin for identification. Two distinctive strains were discovered that differed from any known species of Fusarium (Nirenberg, 1976; Gerlach and Nirenberg, 1982).

Species of *Fusarium* constitute an important component of the mycoflora associated with maize (*Zea mays*) in South and East Africa. Mycotoxins produced by fusaria traditionally classified in the infrageneric grouping called section *Liseola* are associated with

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equine leukoencephalomalacia and pulmonary edema syndrome of swine (Ross et al., 1990), but a connection with human esophageal cancer remains unproven. Besides strains of *F. verticillioides* and *F. subglutinans*, a number of isolates were identified that were diagnosed as a morphologically and phylogenetically distinct species that is described here as *F. pseudoanthophilum*. Further morphological and phylogenetic analysis indicated that these two undescribed *Fusarium* species are members of the *Gibberella fujikuroi* complex (O'Donnell et al., 1998).

MATERIALS AND METHODS

Striga asiatica plants with symptoms (e.g., browning, wilting, rots or lesions) were collected from maize and upland rice fields. Plants were dried by pressing them between sheets of paper at room temperature for several days to preserve them for long-term storage. Infected plants were tested for fungal pathogens by cutting different parts of the leaves, stems and roots into small pieces (ca 5–10 mm). These were surface-sterilized with 70% ethanol for 3 min, rinsed three times in deionized water, dried with filter paper and placed on potato dextrose agar (PDA; Serra, Heidelberg, Germany²; Abbasher et al., 1995). Zea mays kernels were collected from different localities in Zimbabwe.

For examination of colony color, odor and growth rate, strains were grown on PDA (PDA; DIFCO, Detroit, Michigan) under natural day-night illumination at ca 20 C. All microscopic observations were conducted on colonies incubated for 10 to 14 da at 20 C either in complete darkness or under permanent black light (Philips TLD 18 W/08; Nirenberg, 1990) on synthetic low nutrient agar (SNA; per 1 L distilled $H_2O: 1$ g KH_2PO_4 , 1 g KNO_3 , 0.5g $MgSO_4 \cdot 7H_2O$, 0.5g KCl, 0.2 g dextrose, 0.2 g sucrose, 0.6 ml 1N NaOH, 23 g agar) overlaid with a ca 1×2 cm piece of sterile filter paper (Nirenberg, 1990). Measurements and photomicrographs were taken with a Zeiss Axiomat photomicroscope on cultures grown on SNA in complete darkness. At least 30 conidia were measured. The Methuen Handbook of Colour (Kornerup and Wanscher, 1978) was used for the determination of all colors. For additional information on characters used and explanations see Nirenberg and O'Donnell (1998).

DESCRIPTIONS

Fusarium brevicatenulatum Nirenberg, O'Donnell, Kroschel et Andrianaivo, sp. nov. Figs. 1–5

Coloniae in PDA quasi 4.4 mm in dies crescentes, 20 C obscuritate, margine integro. Mycelium aerium albidum, lanosum usque byssaceum. Color in parte aversa initio griseo-aurantiacus, postea atrocaeruleo-griseus. Initium sporulationis in mycelio aerio praecox, conidiis typice in capitulis falsis aggregatis, sub "luce-nigra" continua breviter catenatis. Sporodochia post 10 dies formata. Odor non perceptibilis. Conidiophora in mycelio aerio prostrata, plerumque ex phialidibus constituata, raro unum ramum lateralem ferentia. Phialides conidiophororum in mycelio aerio cylindricae, plerumque monophialidicae, raro polyphialidicae, usque ad 30.0 µm longae et 2.0 µm latae. Conidia in mycelio aerio oblongo-ovalia vel obovoidea, plerumque 0-septata, aliquando 1- vel 2-septata; conidia 0-septata: $(6.0-)6.6-8.1-9.6(-11.8) \times (2.0-)2.2-2.5-2.9(-3.6)$ μm. Conidia sporodochialia rara, falcata, gracilia, recta, usque ad 3-septata: $(28.8-)35.2-39.4-43.5(-45.6) \times (2.8-)$ 3.0-3.4-3.7(-3.9) µm. Chlamydosporae absentes. Holotypi origo geographica. Madagascar, in Striga asiatica. Ex holotypo culturae. NRRL 25446, BBA 69197, CBS 404.97, IMI 375329, DAOM 225122.

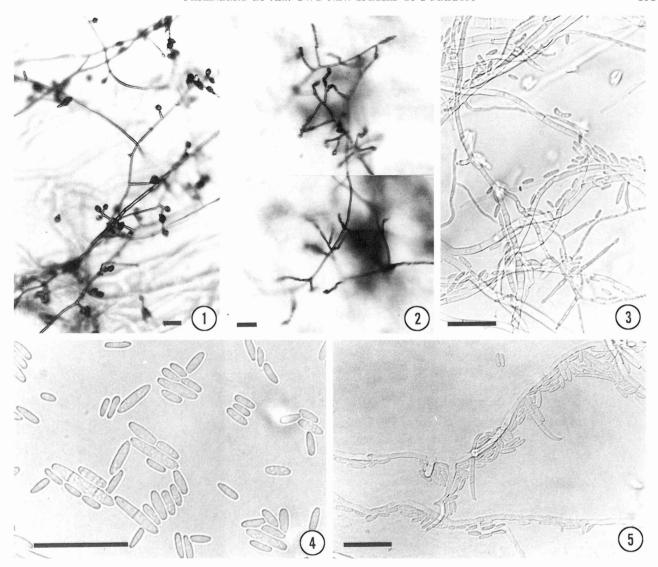
HOLOTYPUS. Depositus in herb. B.

Colonies on PDA showing average mycelial growth rate of 4.4 mm/da at 20 C in the dark; colony margin entire. Aerial mycelium whitish; lanose to fluffy. Pigmentation in reverse greyish orange, becoming dark bluish-gray. Sporulation starting early in the aerial mycelium; conidia typically aggregated in false heads; forming short chains under continuous black light. Sporodochia formed after 10 da. Odor not perceptible. Conidiophores on the aerial mycelium prostrate, mostly identical with phialides, occasionally with one lateral branch. Phialides of conidiophores on the aerial mycelium cylindrical, mostly monophialidic, occasionally polyphialidic, up to 30 µm long and 2.0 µm wide. Conidia borne on the aerial mycelium long-oval to obovoid, mostly 0-septate, sometimes 1- and 2-septate; 0-septate conidia measuring: $(6.0-)6.6-8.1-9.6(-11.8) \times (2.0-)2.2-2.5-2.9$ (-3.6) µm. Conidia borne in sporodochia rare, falcate, slender, straight, up to 3-septate, measuring (28.8-) 35.2-39.4-43.5(-45.6) × <math>(2.8-)3.0-3.4-3.7(-3.9) μm. Chlamydospores absent.

Isolates studied. NRRL 25446 = BBA 69197 = CBS 404.97 = IMI 375329, DAOM 225122 (ex holotype), Madagascar, Striga asiatica; NRRL 25447 = BBA 69198, Madagascar, S. asiatica.

Notes. The epithet refers to the very short chains of catenulate conidia produced on conidiophores in the aerial mycelium under continuous black light. Morphologically *F. brevicatenulatum* is most similar to *F. phyllophilum* Nirenberg et O'Donnell (Nirenberg and O'Donnell, 1998) as both species produce conidia in short chains. However, conidial chains are produced by *F. phyllophilum* primarily in complete darkness,

² Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.



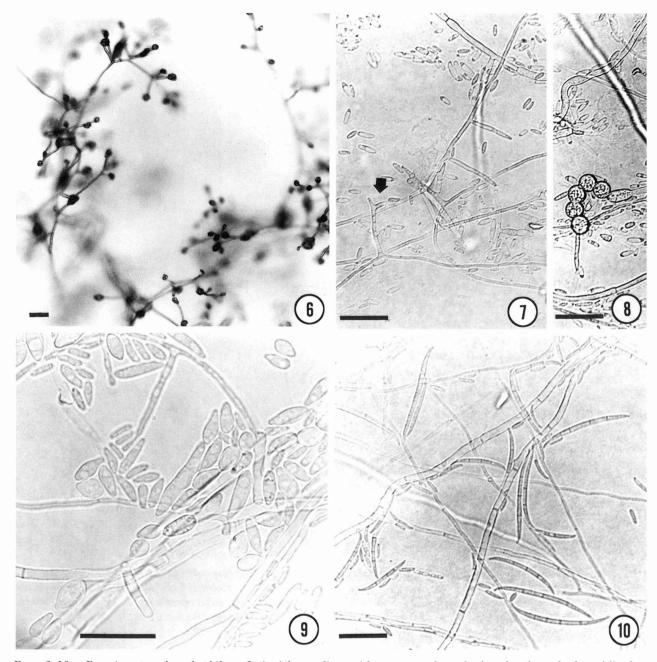
FIGS. 1–5. Fusarium brevicatenulatum. 1. Aerial mycelium with prostrate conidiophores producing conidia in false heads. 2. Aerial mycelium with conidia adhering in very short chains produced under continuous black light. 3. Monophialidic conidiophores of the aerial mycelium. 4. Long oval to obovoid conidia of the aerial mycelium. 5. Two falcate, 3-septate sporodochial conidia with distinctive foot-cells produced under black light. All from BBA 69197. Scale bars = $25 \mu m$.

whereas F. brevicatenulatum forms them only under continuous black light. Morphologically, F. brevicatenulatum would be classified within section Liseola of Fusarium but this infrageneric grouping is not monophyletic (O'Donnell and Cigelnik, 1997). The molecular evidence indicates that F. brevicatenulatum is a member of the African clade of the Gibberella fujikuroi species complex. It is closely related phylogenetically to three other members of this clade (O'Donnell et al., 1998): F. pseudoanthophilum ex Zea mays from Zimbabwe, F. pseudonygamai ex Pennisetum typhoides from Nigeria, and F. verticillioides ex Z. mays and many other agronomically important hosts. Given the host range of its closest known relatives, tests are being conducted to determine whether F. brevicatenulatum is pathogenic to these hosts and to Striga asiatica.

Fusarium pseudoanthophilum Nirenberg, O'Donnell et Mubatanhema, sp. nov. FIGS. 6–10

Coloniae in PDA quasi 3.7 mm in dies crescentes, temperatura 20 C, margine integro. Mycelium aerium aurantiaco-albidum, lanosum. Color in parte aversa pallide aurantiacus vel centro et margine crescente aurantiaco-griseus. Initium sporulationis praecox in mycelio aerio, conidiis in capitulis falsis productis, nonnullis isolatis etiam conidia breviter catenata producentibus. Sporodochia post 3–4 hebdomades producta. Conidiophora mycelii aerii seu erecta seu prostrata; conidiophora altera ex phialidibus constituta, altera modice vel dense ramosa, in 1–3 phialides terminata. Phialides mycelii aerii cylindricae, monophialidicae et polyphialidicae, usque ad 25.0 µm longae et 3.0 µm latae; phialides sporodochiales ampulliformes. Conidia in mycelio aerio obovoidea ad clavata, pyriformia, nonnumquam ob-

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FIGS. 6–10. Fusarium pseudoanthophilum. 6. Aerial mycelium with prostrate, branched and unbranched conidiophores producing conidia in false heads. 7. Simple monophialidic conidiophores, showing a proliferating cell (arrow). 8. Chain of chlamydospores. 9. Obovoid to clavate, and pyriform conidia of the aerial mycelium. 10. Sporodochial conidia produced in complete darkness. FIGS. 6, 7, 9 from BBA 69002, FIG. 8 from BBA 69003, FIG.10 from BBA 69030. Scale bars = $25 \mu m$.

longo-ovalia ad allantoidea, plerumque 0-septata; obovoidea ad clavata $(6.5-)7.6-9.5-11.3(-13.2) \times (2.0-)2.3-2.7-3.1$ (-3.8) µm; pyriformia $(5.8-)6.6-8.0-9.5(-10.2) \times (3.4-)4.0-4.7-5.4(-6.5)$ µm. Conidia sporodochialia falcata, gracilia, cellula apicali acuta et cellula basali pediformi praedita, plerumque 3–5-septata; 3-septata $(40.0-)45.2-50.5-55.9 \times (3.0-)3.3-3.5-3.7(-3.9)$ µm; 5-septata $(59.6-)62.4-66.8-71.2(-74.0) \times 3.5-3.6-3.7(-3.8)$ µm. Chlamydosporae catenatae. Holotypi origo geographica. Gambiza, Zimbabwe, in *Zea mays*. Ex holotypo culturae. NRRL 25211, BBA 69002, Frank 12-KW20, CBS 414.97, IMI 376112.

HOLOTYPUS. Depositus in herb. B.

Colonies on PDA showing an average mycelial growth rate of 3.7 mm/da at 20 C; colony margin entire. Aerial mycelium orange-white; lanose. Pigmentation in reverse pale orange, orange-grey at the center and growing margin. Sporulation starting early in the aerial mycelium; conidia produced in false heads, some isolates also producing conidia in short chains. Sporodochia produced after 2 to 4 wk. Conidiophores of the aerial mycelium erect or pros-

trate; conidiophores either identical with phialides or moderately to strongly branched, terminating in 1-3 phialides. Phialides of the aerial mycelium cylindrical, monophialidic or polyphialidic, to 25.0 µm long and 3.0 µm wide; sporodochial phialides flaskshaped. Conidia borne in the aerial mycelium obovoid to clavate, pyriform, sometimes long-oval to allantoid, mostly 0-septate, obovoid to clavate, measuring: $(6.5-)7.6-9.5-11.3(-13.2) \times (2.0-)2.3-2.7-3.1$ (-3.8) µm; pyriform (5.8-)6.6-8.0-9.5(-10.2) × (3.4-)4.0-4.7-5.4(-6.5) µm. Conidia borne in sporodochia falcate, slender with a pointed apical cell and a foot cell, mostly 3–5-septate; 3-septate: (40.0–) $45.2-50.5-55.9 \times (3.0-)3.3-3.5-3.7(-3.9) \mu m; 5-sep$ tate: $(59.6-)62.4-66.8-71.2(-74.0) \times 3.5-3.6-3.7$ (-3.8) µm. Chlamydospores produced in chains.

Isolates studied. NRRL 25206 = BBA 69030 = CBS 745.97 = IMI 375340 = DAOM 225134 = Frank GW23, Gweru, Zimbabwe, Zea mays; NRRL 25209 = BBA 69003 = CBS 415.97 = Frank KA18, Karoi, Zimbabwe, Z. mays; NRRL 25210 = BBA 69004 = IMI 360543 = Frank GW24, Gweru, Zimbabwe, Z. mays; NRRL 25211 = BBA 69002 = CBS 414.97 = IMI 376112 = Frank 12-KW20 (ex holotype), Gambiza, Zimbabwe, Z. mays.

Notes. The epithet refers to the morphological similarity of F. pseudoanthophilum to F. anthophilum. Both species produce pyriform conidia on branched conidiophores from mono- and polyphialides (Gerlach and Nirenberg, 1982; Nelson et al., 1983). Fusarium pseudoanthophilum also forms very short chains of conidia under continuous black light and chlamydospores in chains, features never seen in F. anthophilum. Phylogenetically, F. pseudoanthophilum is closely related to the following species within the African clade of the Gibberella fujikuroi complex (O'Donnell and Cigelnik, 1997; O'Donnell et al., 1998): F. brevicatenulatum, F. pseudonygamai O'Donnell & Nirenberg (Nirenberg and O'Donnell, 1998), and F. verticillioides (Sacc.) Nirenberg (Nirenberg, 1976). Isolates of F. pseudoanthophilum tested by Mubatanhema (1994) did not produce moniliformin, fumonisin B1, or zearalenone.

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